

Morphological and phylogenetic resolution of *Arthrinium* from medicinal plants in Yunnan, including *A. cordylines* and *A. pseudomarii* spp. nov.

TONG-ZHENG CHEN^{1^}, YAN ZHANG^{2,3,4^}, XIAO-BING MING¹,
QIAN ZHANG¹, HUI LONG¹, KEVIN D. HYDE⁵, YAN LI^{6^A}, YONG WANG^{1^B}

¹ Department of Plant Pathology, Agriculture College, Guizhou University,
Guiyang, Guizhou 550025, China

² Beijing University of Agriculture, Beijing 102206 China

³ Beijing Bei Nong Enterprise Management Co., Ltd., Beijing, 102206, China

⁴ Beijing Forestry University, Beijing, 100083, China

⁵ Center of Excellence in Fungal Research and School of Science, Mae Fah Luang University,
Chiang Rai, 57100, Thailand

⁶ Guizhou Key Laboratory Agro-Bioengineering, Guizhou University,
Guiyang, Guizhou 550025, China

* CORRESPONDENCE TO: ^A166997@qq.com ^Byongwangbis@aliyun.com

ABSTRACT—Twenty-one strains of *Arthrinium* were cultured from leaf samples of ten medicinal plant hosts in Yunnan Province, China. Morphological and multi-locus ITS+TUB+TEF1 sequence analyses revealed that the strains represented two previously described species (*A. paraphaeospermum* and *A. rasikravindrae*) and two new species: *Arthrinium cordylines*, which produces subglobose conidia that are shorter and wider than *A. aureum* but larger than *A. hydei*, and *Arthrinium pseudomarii*, which produces subglobose to ellipsoid conidia narrower than *A. hispanicum*, *A. marii*, and *A. mediterranei*.

KEY WORDS—*Apiosporaceae*, phylogenetic analysis, taxonomy, *Xylariales*

Introduction

Arthrinium (*Apiosporaceae*, *Xylariales*, *Sordariomycetes*) is widely distributed around the world (Hyde & al. 2020). Its species often occur on different plant

[^] Tong-Zheng Chen and Yan Zhang contributed equally to this paper.

substrates as saprobes (Agut & Calvo 2004, Singh & al. 2013, Dai & al. 2016), pathogens (Martínez-Cano & al. 1992, Thangaraj & al. 2019), and endophytes (Ramos & al. 2010, Huang & Jiang 2015, Rashmi et al. 2019). *Arthrinium* is morphologically distinguished from other asexual genera by its basauxic conidiophores with terminal and intercalary polyblastic conidiogenous cells (Hughes 1953, Minter 1985, Hyde & al. 1998, Wijayawardene & al. 2016). However, it is impossible to resolve taxonomy within the genus without molecular data (Crous & Groenewald 2013).

During this study, we isolated 21 *Arthrinium* strains from leaves of ten different medicinal plants in Yunnan Province, China. Morphological and ITS + TUB + TEF1 sequence analyses clarified their taxonomic placements, revealing two previously described species (*A. paraphaeospermum* and *A. rasikravindrae*) and two new species, here proposed as *A. cordylines* and *A. pseudomarii*.

Materials & methods

Culture & morphology

Arthrinium strains were isolated from leaf samples collected from Yunnan Province, P.R. China, using the 'single-spore method' (Senanayake et al. 2020). Colonies were transferred to oat-agar (OA) and incubated at room temperature (c. 28 °C). Following 2–3 weeks of incubation, morphological characters were recorded. Conidial and conidiophore morphologies were examined using an Olympus BX53 compound microscope. The holotype specimens are deposited in the Herbarium of Department of Plant Pathology, Agricultural College, Guizhou University, China (HGUP), and pure cultures are deposited in the Department of Plant Pathology Culture Collection, Agriculture College, Guizhou University, China (GUCC), and isotype cultures of *Arthrinium cordylines* and *A. pseudomarii* are conserved in Guizhou Culture Collection (GZCC), Guizhou, China.

DNA amplification & sequencing

DNA amplification, sequencing and phylogenetic analysis follow Dissanayake & al. (2020) with modifications. Fungal cultures were grown on OA medium at 28 °C. When nearly covering the whole Petri-dish (90 mm diam), fresh mycelia were scraped from the surface with sterilized scalpels. Genomic DNA was extracted using a Biomiga Fungus Genomic DNA Extraction Kit (GD2416), following the manufacturers protocol. DNA was amplified in 25 µL reactions containing 2.5 µL 10 × PCR buffer, 1 µL of each primer (10 µM), 1 µL template DNA, and 0.25 µL Promega Taq DNA polymerase. Primers ITS4 and ITS5 (White & al. 1990) were used to amplify the ITS region, and Two protein-coding gene fragments (TUB and TEF1) were amplified by the primers T1/Bt2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997) and EF1-728F/EF-2 (O'Donnell & al. 1998, Carbone & Kohn 1999).

TABLE 1 Sequences of *Arthrinium* spp. and *Nigrospora gorlenkoana* outgroup used for phylogenetic analyses

SPECIES	STRAIN	GENBANK ACCESSIONS		
		ITS	TUB	TEF1
<i>A. arundinis</i>	CBS 114316	KF144884	KF144974	KF145016
<i>A. aureum</i>	CBS 244.83*	AB220251	KF144981	KF145023
<i>A. bambusae</i>	LC7106*	KY494718	KY705186	KY806204
<i>A. camelliae-sinensis</i>	LC5007*	KY494704	KY705173	KY705103
<i>A. caricicola</i>	AP23518	MK014871	MK017977	MK017948
<i>A. cordyline</i>	GUCC 10026	MT040105	MT040147	MT040126
	GUCC 10027*	MT040106	MT040148	MT040127
<i>A. curvatum</i> var. <i>minus</i>	AP25418	MK014872	MK017978	MK017949
<i>A. descalsii</i>	AP31118A*	MK014870	MK017976	MK017947
<i>A. dichotomanthi</i>	LC4950*	KY494697	KY705167	KY705096
<i>A. esporlense</i>	AP16717*	MK014878	MK017983	MK017954
<i>A. euphorbiae</i>	IMI 285638b	AB220241	AB220288	—
<i>A. garethjonesii</i>	HKAS 96289*	NR_154736	—	—
	JHB004	KY356086	—	—
<i>A. guizhouense</i>	LC5318	KY494708	KY705177	KY705107
	LC5322*	KY494709	KY705178	KY705108
<i>A. gutiae</i>	CBS 135835	KR011352	KR011350	KR011351
<i>A. hispanicum</i>	IMI 326877*	AB220242	AB220289	—
<i>A. hydei</i>	CBS 114990*	KF144890	KF144982	KF145024
	JHB0012	KY356087	—	—
	LC7103	KY494715	KY705183	KY705114
	LC7105	KY494717	KY705185	KY705116
<i>A. hyphopodii</i>	MFLUCC 15-0003*	KR069110	—	—
<i>A. hysterinum</i>	AP2410173	MK014876	—	—
<i>A. ibericum</i>	AP10118*	MK014879	MK017984	MK017955
<i>A. italicum</i>	AP221017*	MK014880	MK017985	MK017956
<i>A. japonicum</i>	IFO 30500	AB220262	AB220309	—
<i>A. jatrophae</i>	CBS 134262	NR_154675	—	—
	MMI 00052*	JQ246355	—	—
<i>A. jiangxiense</i>	LC4577*	KY494693	KY705163	KY705092
<i>A. kogelbergense</i>	CBS 113333*	KF144892	KF144984	KF145026
<i>A. longistromum</i>	MFLUCC 11-0481*	KU940141	—	—
	MFLUCC 11-0479*	KU940142	—	—
	MFLU 15-1184*	NR_154716	—	—
<i>A. malaysianum</i>	CBS 102053*	KF144896	KF144988	KF145030
<i>A. marii</i>	CBS 497.90*	AB220252	KF144993	KF145035
<i>A. mediterranei</i>	IMI 326875*	AB220243	AB220290	—
<i>A. mytilomorphum</i>	DAOM 214595*	KY494685	—	—
<i>A. neosubglobosum</i>	HKAS 96354	NR_154737	—	—
	JHB006	KY356089	—	—
	JHB007*	KY356090	—	—

<i>A. obovatum</i>	LC4940*	KY494696	KY705166	KY705095
<i>A. ovatum</i>	CBS 115042*	KF144903	KF144995	KF145037
<i>A. paraphaeospermum</i>	MFLUCC 13-0644*	KX822128	–	–
	GUCC 10124	MT040108	MT040150	MT040129
	GUCC 10125	MT040109	MT040151	MT040130
	GUCC 10126	MT040110	MT040152	MT040131
	GUCC 10127	MT040111	MT040153	MT040132
	GUCC 10128	MT040112	MT040154	MT040133
	GUCC 10129	MT040113	MT040155	MT040134
<i>A. phaeospermum</i>	CBS 114314	KF144904	KF144996	KF145038
<i>A. phragmitis</i>	CPC18900*	KF144909	KF145001	KF145043
<i>A. piptatheri</i>	AP4817A*	MK014893	–	MK017969
<i>A. pseudomarii</i>	GUCC 10214	MT040114	MT040156	MT040135
	GUCC 10221	MT040115	MT040157	MT040136
	GUCC 10225	MT040116	MT040158	MT040137
	GUCC 10263	MT040117	MT040159	MT040138
	GUCC 10254	MT040118	MT040160	MT040139
	GUCC 10259	MT040119	MT040161	MT040140
	GUCC 10260	MT040120	MT040162	MT040141
	GUCC 10270	MT040121	MT040163	MT040142
	GUCC 10226	MT040122	MT040164	MT040143
	GUCC 10227	MT040123	MT040165	MT040144
	GUCC 10228*	MT040124	MT040166	MT040145
	GUCC 10261	MT040125	MT040167	MT040146
<i>A. pseudoparenchymaticum</i>	LC7234*	KY494743	KY705211	KY705139
<i>A. pseudosinense</i>	CPC 21546*	KF144910	–	KF145044
<i>A. pseudospegazzinii</i>	CBS 102052*	KF144911	KF145002	KF145045
<i>A. pterospermum</i>	CPC 20193*	KF144913	KF145004	KF145046
<i>A. puccinioides</i>	AP26418	MK014894	MK017998	MK017970
	CBS 549.86	AB220253	AB220300	–
<i>A. rasikravindrae</i>	LC5449	KY494713	KY705182	KY705112
	LC8179	KY494759	KY705227	KY705155
	NFCCI 2144*	JF326454	–	–
	GUCC 10088	MT040107	MT040149	MT040128
<i>A. sacchari</i>	CBS 212.30	KF144916	KF145005	KF145047
<i>A. saccharicola</i>	CBS 191.73	KF144920	KF145009	KF145051
<i>A. serenense</i>	IMI 326869*	AB220250	AB220297	–
<i>A. sporophleum</i>	AP21118	MK014898	MK018001	MK017973
<i>A. subglobosum</i>	MFLUCC 11-0397*	KR069112	–	–
<i>A. subroseum</i>	LC7292*	KY494752	KY705220	KY705148
<i>A. thailandicum</i>	MFLUCC 15-0202*	KU940145	–	–
<i>A. xenocordella</i>	CBS 478.86*	KF144925	KF145013	KF145055
<i>A. yunnanum</i>	MFLUCC 15-0002*	KU940147	–	–
<i>N. gorlenkoana</i>	CBS 480.73	KX986048	KY019456	KY019420

* = ex-type strains. Strains and sequences generated in this study are shown in **bold**

The annealing temperatures were adjusted to 52 °C for ITS and TUB, and 56 °C for TEF1. The PCR amplicons were purified and sequenced by SinoGenoMax. The DNA sequences were submitted to GenBank (TABLE 1). The DNA base differences of different gene loci between our strains and ex-type strains of related taxa are shown in TABLE 2.

Phylogenetic analyses

DNA sequences from our strains and reference sequences downloaded from GenBank (Crous & Groenewald 2013, Dai & al. 2016, Wang & al. 2018, Pintos & al. 2019) were used for maximum parsimony (MP) and maximum likelihood (ML) analyses. Sequences in the order of ITS+TUB +TEF1 were aligned using an online version of MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/>). Ambiguous

TABLE 2. DNA base differences in three gene regions comparing our *Arthrinium* sequences and sequences from related species.

Species	Strain	ITS (611)	TUB (752)	TEF1 (464)
<i>Arthrinium cordyline</i>	GUCC 10026 [*]	0	0	0
	GUCC 10027	0	0	0
<i>A. hydei</i>	CBS 114990 [*]	3	15	13
<i>A. aureum</i>	CBS 244.83 [*]	3	24	19
<i>A. rasikravindrae</i>	GUCC 10088	0	0	0
	LC5449	1	4	0
<i>A. paraphaeospermum</i>	GUCC 10125	0	0	0
	GUCC 10124	15	0	0
	GUCC 10126	0	0	1
	GUCC 10127	0	0	0
	GUCC 10128	0	0	0
	GUCC 10129	0	0	3
	MFLU 13-0644 [*]	0	\	\
<i>A. pseudomarii</i>	GUCC 10228 [*]	0	0	0
	GUCC 10214	1	0	2
	GUCC 10221	0	2	2
	GUCC 10225	1	0	2
	GUCC 10263	1	0	2
	GUCC 10254	1	0	0
	GUCC 10259	1	0	0
	GUCC 10260	1	0	0
	GUCC 10270	1	0	0
	GUCC 10226	1	0	0
	GUCC 10227	0	0	1
	GUCC 10261	0	1	1
<i>A. hispanicum</i>	IMI 326877	1	2	\
<i>A. marii</i>	CBS 497.90	2	2	12
<i>A. mediterranei</i>	IMI 326875	1	2	\

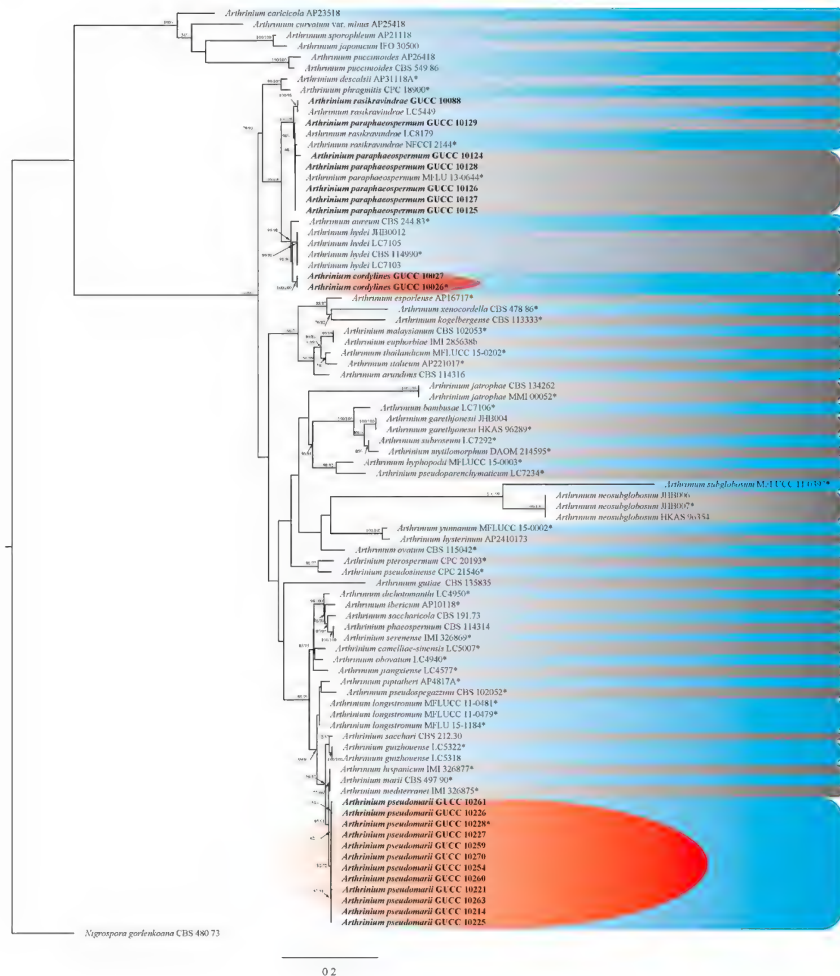


Fig. 1. Phylogenetic tree of *Arthrinium* spp., based on combined ITS, TUB, and TEF1 sequence alignments generated from maximum likelihood and maximum parsimony analyses. Bootstrap support values >50% are given at the nodes (ML/MP). The tree is rooted with *Nigrospora gorklenkoana* CBS 480.73. Ex-type strains are indicated with *. Bold entries represent newly sequenced strains. The new species are shown in bold and on a red background.

regions were excluded from the analyses, and gaps were treated as missing data. MP analyses were performed in PAUP v. 4.0b10 (Swofford 2002), using the heuristic search option with 1,000 random taxa addition and tree bisection and re-connection (TBR) as the branch swapping algorithm. Maxtrees = 5000 was

set to upbuild the phylogenetic tree. Tree Length (TL), Consistency Indices (CI), Retention Indices (RI), Rescaled Consistency Indices (RC), and Homoplasy Index (HI) were calculated for each tree. CIPRES Science Gateway (<https://www.phylo.org/portal2/login.action>) used the resulting Phylip file to generate the ML tree with GTRGAMMA as the nucleotide substitution model and RAXML-XSEDE with 1000 bootstrap inferences. DNA sequences generated with forward and reverse primers were used to obtain consensus sequences using MEGA v. 6.06 (Tamura & al. 2013). Sequences were aligned using MAFFT v7.307 online version (Katoh & Standley 2016) and edited by hand using MEGA v. 5.1.

Phylogenetic analytical results

Strain sequences comprised 480–633 bp (ITS), 460–477 bp (TEF1), and 701–829 bp (TUB) in length. Following alignment, the combined ITS+TUB+TEF1 dataset contained 2096 characters (ITS: 1–711 + TUB: 714–1246 + TUB: 1249–2096, adding two “N’s” after the ITS and TEF1 sequences to distinguish between the two gene regions), of which 876 were parsimony-informative characters. Sequences from 50 *Arthrinium* species (representing 63 strains) were downloaded, plus the outgroup *Nigrospora gorkeniana* Novobr. (CBS 480.73). Five thousand equally most parsimonious trees were obtained (TL = 3509, CI = 0.54, RI = 0.82, RC = 0.44, and HI = 0.46). Similar topologies were obtained by MP and ML methods, and the first equally most parsimonious tree was selected to display the phylogenetic relationship of *Arthrinium* species (FIG. 1). In FIG. 1, our strains clustered into three subclades: (1) GUCC 10026, GUCC 10027, *A. aureum* Calvo & Guarro, and *A. hydei* Crous; (2) GUCC 10214, GUCC 10221, GUCC 10225–10228, GUCC 10254, GUCC 10259–10261, GUCC 10263, and GUCC 10270 originated from five medicinal plants and formed a subclade with *A. hispanicum* Larrondo & Calvo, *A. marii* Larrondo & Calvo, and *A. mediterranei* Larrondo & Calvo; and (3) GUCC 10088 and GUCC 10124–10129 were adjacent to *A. paraphaeospermum* and *A. rasikravindrae*. All subclades are supported with high MP and ML bootstrap values (FIG. 1).

Taxonomy

Arthrinium cordyline T.Z. Chen, Yong Wang bis & K.D. Hyde, sp. nov.

FIG. 2

MB 834522

Differs from *Arthrinium aureum* by its shorter but wider conidia; and from *A. hydei* by its larger subglobose to ellipsoid conidia.

TYPE: China, Yunnan Province, Xichuangbanna, from leaves of *Cordyline fruticosa* (L.) A. Chev. (*Asparagaceae*), 20.10.2018, leg. Y. Wang, (Holotype, HGUP 10027 [dried colony on OA]; ex-type culture, GUCC 10027; isotype culture, GZCC21-0020).

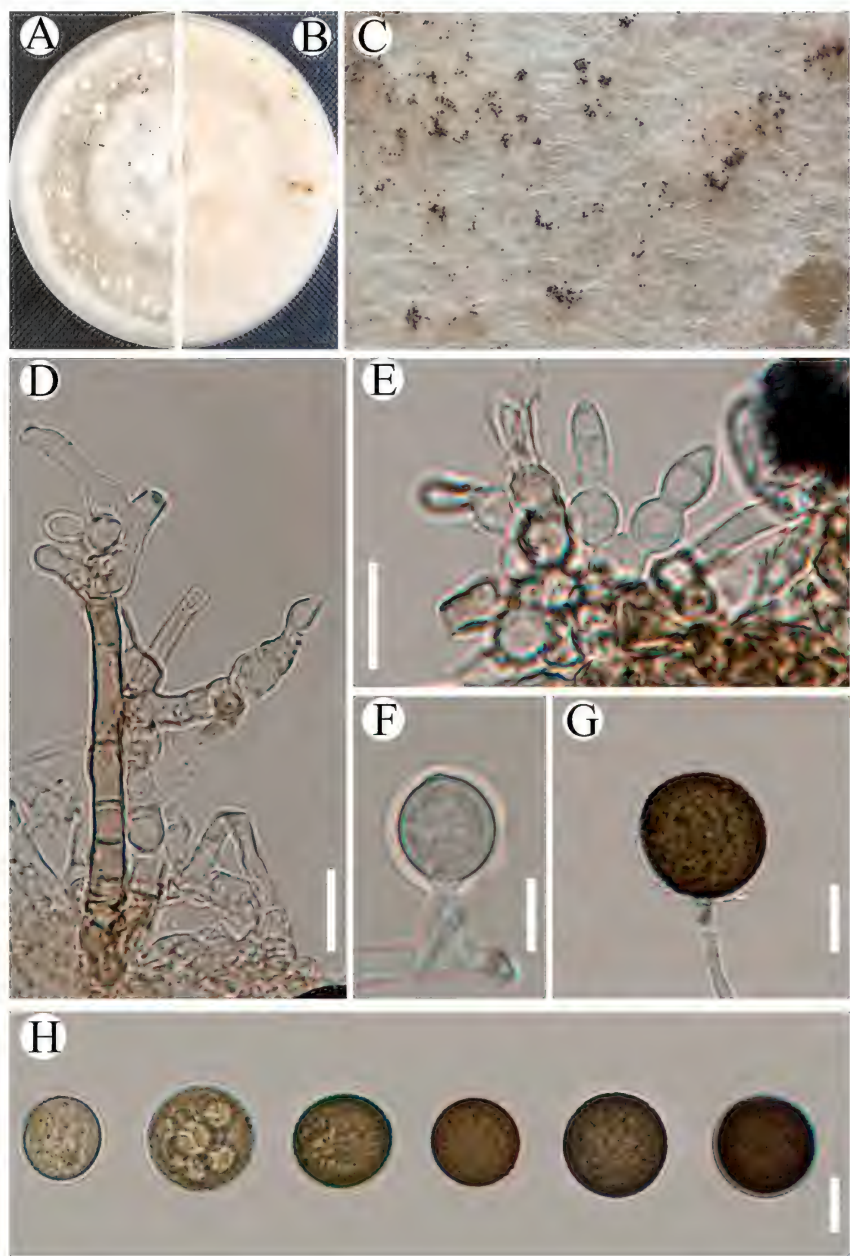


FIG. 2. *Arthrinium cordylines* (ex-holotype GUCC 10027). A. Host (*Cordyline fruticosa*) and symptoms; B, C. Cultures on OA; D. Colony on OA producing conidial masses; E–H. Conidiogenous cells giving rise to conidia; I Conidia. Scale bars: E, F, H, I = 10 µm; G = 5 µm.

ETYMOLOGY: The specific epithet refers to the host *Cordyline fruticosa*, from which the fungus was isolated.

HYPHAE hyaline, septate, branched, smooth, 1.5–2.5 μm diam. **CONIDIOPHORES** reduced to conidiogenous cells. **CONIDIOGENOUS CELLS** erect, aggregated into clusters on hyphae, hyaline to pale brown, smooth, doliiform to ampulliform or lageniform, (3–)5–10(–15) $\times$ 2.6–5.3 μm (av. $7.0 \times 4.5 \mu\text{m}$, $n = 30$). **CONIDIA** olivaceous to brown, smooth to finely roughened, subglobose to ellipsoid, 15–19 $\times$ 12.5–18.5 μm (av. $17.5 \times 15.7 \mu\text{m}$, $n = 30$).

COLONIES on OA flat, spreading, circular, smooth at margin, with abundant aerial mycelia, surface and reverse white to grey, sometimes with light pink.

TELEOMORPH: not observed.

ADDITIONAL MATERIAL EXAMINED: CHINA: YUNNAN PROVINCE, Xichuangbanna, from leaves of *Cordyline fruticosa*, Y. Wang (HGUP 10026).

NOTES—Our two strains (GUCC 10026, GUCC 10027) formed a branch with the ex-type strains of *A. aureum* (CBS 244.83) and *A. hydei* (CBS 114990) with 99% (MP) and 98% (ML) bootstrap support. *Arthrinium cordyline* also showed a close relationship to *A. hydei* (91% MP/80% ML). DNA pair-wise comparisons revealed three similar ITS, 13 similar TEF1, and 15 similar TUB base pairs, confirming that *A. cordyline* and *A. hydei* are separate species according to the guidelines of Jeewon & Hyde (2016). Morphologically, conidia in *A. cordyline* are shorter and wider than *A. aureum* (14–30 $\times$ 10–15 μm ; Calvo & Guarro 1980) but larger than the globose conidia of *A. hydei* (11–12 μm diam; Crous & Groenewald 2013).

Arthrinium pseudomarii T.Z Chen, Yong Wang bis & K.D. Hyde, sp. nov. FIG. 3
MB 834523

Differs from *Arthrinium hispanicum*, *A. marii*, and *A. mediterranei* by its narrower subglobose to ellipsoid conidia.

TYPUS: China, Yunnan Province, Kunming Institute of Botany, from leaves of *Aristolochia debilis* Siebold & Zucc. (*Aristolochiaceae*), 15.1.2018, leg. X.B. Ming (**Holotype**, HGUP 10228 [dried colony on OA]; ex-type culture, GUCC 10228; isotype culture, GZCC21-0021).

ETYMOLOGY: The specific epithet refers to the related species *Arthrinium marii*.

HYPHAE hyaline, septate, branched, smooth, 2–3 μm diam. **CONIDIOPHORES** basauxic, mononematous, macronematous. **CONIDIOGENOUS CELLS** erect, aggregated into clusters on hyphae, hyaline to pale brown, smooth, doliiform to ampulliform, or lageniform, 8–13 $\times$ 2.5–5 μm (av. $6.0 \times 4 \mu\text{m}$, $n = 30$).

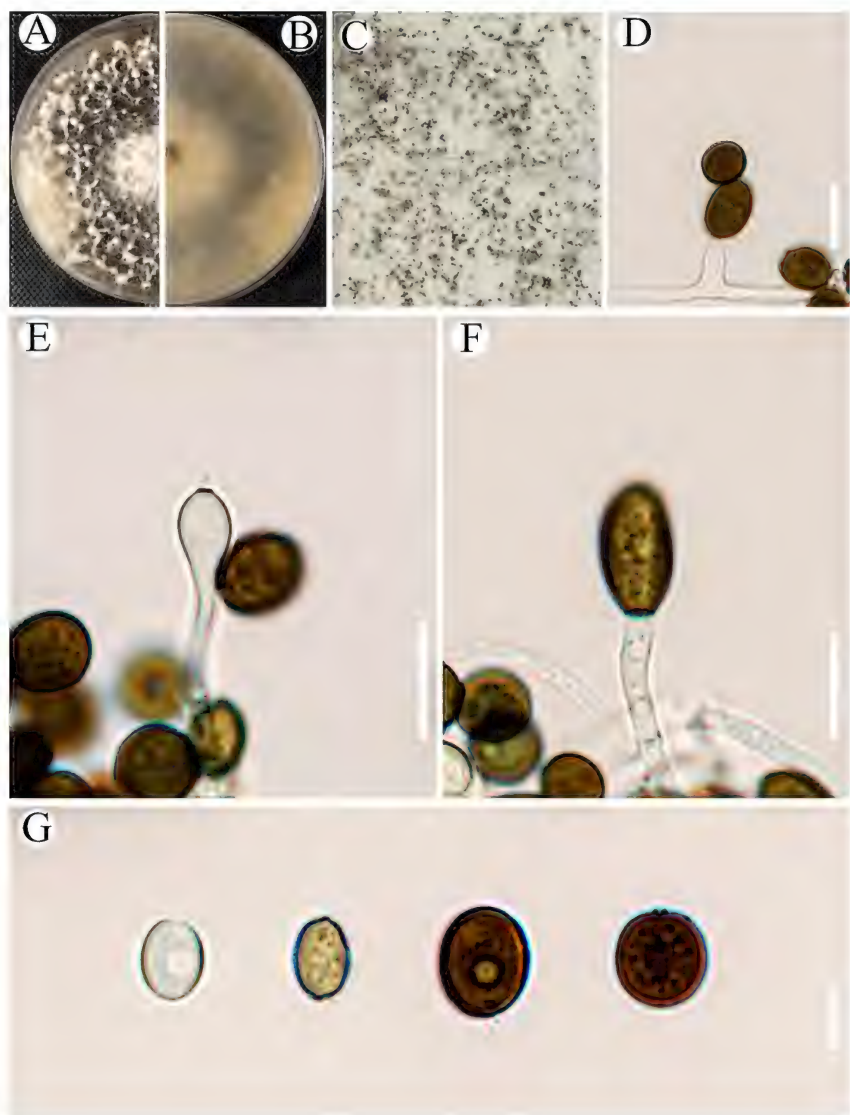


FIG. 3. *Arthrimum pseudomarii* (ex-holotype GUCC 10228). A. Host (*Aristolochia debilis*) and symptoms; B, C. Cultures on OA; D. Colony on OA producing conidial masses; E–G. Conidiogenous cells giving rise to conidia; H. Conidia. Scale bars: E–G = 20 μ m; H = 5 μ m.

CONIDIA solitary, lateral or terminal, olivaceous to brown, smooth to finely roughened, subglobose to ellipsoid, 6–9 $\times$ 4.5–6 μ m (av. 7.7 $\times$ 5 μ m, n = 30).

COLONIES on OA, flat, spreading, margin circular, with abundant aerial mycelia, surface and reverse white to grey.

TELEOMORPH: not observed.

ADDITIONAL MATERIAL EXAMINED: CHINA: YUNNAN PROVINCE, Kunming Institute of Botany, from leaves of *Pachysandra terminalis* Siebold & Zucc. (Buxaceae), Q. Zhang (GUCC 10214); from leaves of *Disporopsis pernyi* (Hua) Diels (Asparagaceae), H. Long (GUCC 10221); from leaves of *Aristolochia debilis*, X.B. Ming (GUCC 10225, GUCC 10226, GUCC 10227, GUCC 10261); from leaves of *Cordyline fruticosa*, X.B. Ming (GUCC 10263); from leaves of *Lycium chinense* Mill. (Solanaceae), X.B. Ming (GUCC 10254), from leaves of *Hosta ventricosa* Stearn (Asparagaceae), X.B. Ming (GUCC 10259, GUCC 10260); from leaves of *Epimedium acuminatum* Franch. (Berberidaceae), X.B. Ming (GUCC 10270).

NOTES—The twelve *Arthrinium pseudomarii* strains formed a relatively independent branch with 82% MP/72% ML support, which showed a close relationship to *A. hispanicum*, *A. marii*, and *A. mediterranei* with 95% MP/93% ML bootstrap support. We compared the DNA base pairs for three gene regions in the subclade (our twelve strains, *A. hispanicum*, *A. marii*, and *A. mediterranei*) which revealed that for *A. marii* there are 14–16 TEF1, 0–2 ITS, and 1–3 TUB differences (TABLE 2). There were 0–2 ITS base pair differences separating *A. hispanicum* and *A. mediterranei*. Morphologically, conidia in *A. pseudomarii* are narrower than conidia in *A. hispanicum* ($7.5\text{--}8.5 \times 6.2\text{--}7.6\ \mu\text{m}$), *A. marii* ($7.2\text{--}7.5 \times 6.1\text{--}6.5\ \mu\text{m}$), and *A. mediterranei* ($9\text{--}9.5 \times 7.5\text{--}9\ \mu\text{m}$) (Larrondo & Calvo 1990, 1992).

Arthrinium paraphaeospermum Senan. & K.D. Hyde,

Fungal Diversity 80: 198 (2016).

FIG. 4

HYPHAE hyaline, septate, branched, 3–5 μm diam. CONIDIOPHORES reduced to conidiogenous cells. CONIDIOGENOUS CELLS erect, aggregated into clusters on hyphae, hyaline to pale brown, smooth, elongated, conical to ampulliform, 15–30 $\times$ 3–5 μm (av. 19 $\times$ 4 μm , $n = 30$). CONIDIA olivaceous to brown, smooth to finely roughened, mostly subglobose, 9–15 μm (av. 13 μm , $n = 30$) diam.

COLONIES on OA, flat, spreading, circular, smooth at margin with abundant aerial mycelia, surface and reverse white to grey, sometimes with light pink.

TELEOMORPH: not observed.

MATERIAL EXAMINED: CHINA, YUNNAN PROVINCE, Kunming Institute of Botany, from leaves of *Epimedium sagittatum* (Siebold & Zucc.) Maxim. (Berberidaceae), 15.1.2018, leg. X.B. Ming (GUCC 10124–10127); from leaves of *Neocheiropteris palmatopedata* (Baker) Christ (Polypodiaceae), 15.1.2018, leg. X.B. Ming (GUCC 10128, GUCC 10129).

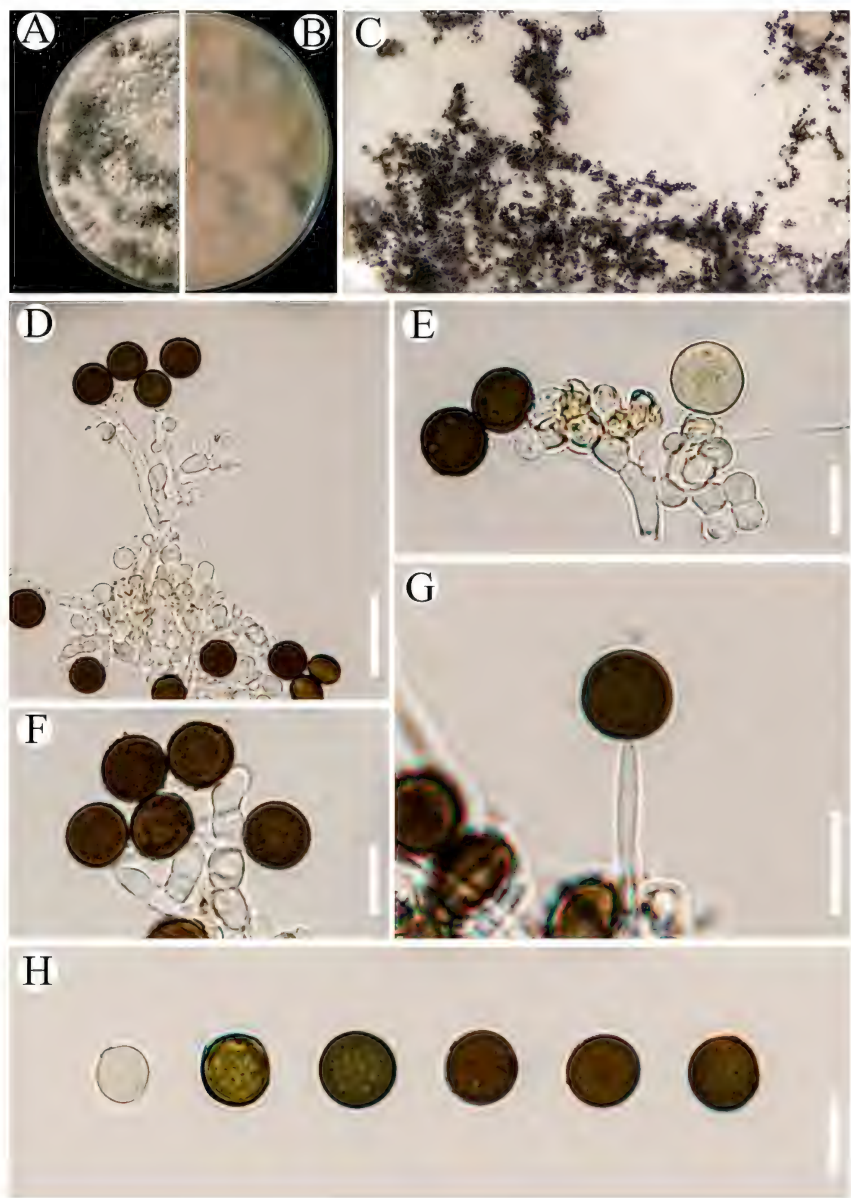


FIG. 4. *Arthrinium paraphaeospermum* (GUCC 10125). A. Host (*Epimedium sagittatum*) and symptoms; B, C. Cultures on OA; D. Colony on OA producing conidial masses; E–H. Conidiogenous cells giving rise to conidia; I. Conidia. Scale bars: E = 20 μm; E–I = 10 μm.

NOTES—Phylogenetic group the six strains cited above with *A. paraphaeospermum* and *A. rasikravindrae*. Morphological comparison indicated that our strains were more similar to *A. paraphaeospermum*, in that the subglobose conidia differed from the dimorphous conidia of *A. rasikravindrae* (lenticular, ovoid: $10\text{--}15 \times 6\text{--}10.5\ \mu\text{m}$; elongate to clavate: $15\text{--}25 \times 7.5\text{--}10\ \mu\text{m}$; Singh & al. 2013, Hyde & al. 2016) and the subglobose to mainly ovoid conidia ($8\text{--}13 \times 6.5\text{--}11.5\ \mu\text{m}$) of GUCC 10088. Although GUCC 10124 showed 15 DNA base differences in the ITS region, the TUB and TEF1 sequences were nearly the same for all five strains (TABLE 2). Thus, we assign these collections to *A. paraphaeospermum*.

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FIG. 5

HYPHAE hyaline, septate, branched, smooth, $2\text{--}3\ \mu\text{m}$ diam. CONIDIOPHORES reduced to conidiogenous cells. CONIDIOGENOUS CELLS erect, aggregated in clusters on hyphae, hyaline to pale brown, smooth, doliiform to ampulliform, or lageniform, $10\text{--}20 \times 3.5\text{--}5\ \mu\text{m}$ (av. $14.0 \times 4.0\ \mu\text{m}$, $n = 30$). CONIDIA arising acropleurogenously, olivaceous to brown, smooth to finely roughened, subglobose to mainly ovoid, $8\text{--}13 \times 6.5\text{--}11.5\ \mu\text{m}$ (av. $10.5 \times 10\ \mu\text{m}$, $n = 30$).

COLONIES on OA, flat, spreading, margin circular, with abundant aerial mycelia, surface and reverse white to grey, sometimes with light pink.

TELEOMORPH: not observed.

MATERIAL EXAMINED: CHINA, YUNNAN PROVINCE, Kunming Institute of Botany, from leaves of *Aspidistra elatior* Blume (*Asparagaceae*), 15.1.2018, leg. X.B. Ming (GUCC 10088).

NOTES—Phylogenetic analyses (FIG. 1) confirmed that strain GUCC 10088 was closely related to *A. rasikravindrae* with high bootstrap support (MP = 100% / ML = 93%). DNA base pair comparison (TABLE 2) revealed only one ITS base pair difference as compared to representative culture of *A. rasikravindrae* (LC 5449), and no differences in the TUB and TEF1 sequences. Morphological comparison also indicated that GUCC 10088 represented *A. rasikravindrae* (Singh & al. 2013).

Discussion

Our regional survey of *Arthrinium* diversity in Yunnan Province revealed that *Arthrinium* was not host-specific, because the same species could be isolated from different plants (e.g., *A. paraphaeospermum* and *A. pseudomarii*), and strains of these two species possessed a very high base similarity in



FIG. 5. *Arthrinium rasikravindrae* (GUCC 10088). A. Host (*Aspidistra elatior*) and symptoms; B, C. Cultures on OA; D. Colony on OA producing conidial masses; E–I. Conidiogenous cells giving rise to conidia; J, K. Conidia. Scale bars: E, G = 10 μ m; F, H–K = 5 μ m.

different gene regions, which was consistent with Crous & Groenewald (2013) and Wang & al. (2018). Secondly, the discovery of four taxa suggests that *Arthrinium* is highly diverse in southwestern China, which would parallel the rich phyto-floristic diversity in this region. We also confirmed that morphological comparison alone is sufficient to identify *Arthrinium* taxa, and that sequence analysis with base comparison is essential to resolve species.

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